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Physiological intensity profile, exercise load and performance predictors of a 65-km mountain ultra-marathon

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1 **Physiological intensity profile, exercise load and performance predictors of a 65-km Mountain**
2 **Ultra-Marathon**

3 Alessandro Fornasiero ^{1,2}, Aldo Savoldelli ^{1,2}, Damiano Fruet ¹, Gennaro Boccia ^{1,2,3}, Barbara
4 Pellegrini ^{1,2}, Federico Schena ^{1,2}

5 ¹ *CeRiSM, Sport Mountain and Health Research Center, University of Verona, Rovereto, Italy*

6 ² *Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona,*
7 *Verona, Italy*

8 ³ *NeuroMuscularFunction research group, School of Exercise and Sport Sciences, Department of*
9 *Medical Sciences, University of Turin, Turin, Italy*

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21 **Corresponding Author**

22 Alessandro Fornasiero, CeRiSM, Sport, Mountain and Health Research Center, University of
23 Verona, via Matteo del Ben, 5/b, 38068 Rovereto, Italy

24 Tel: +39 0464483511; Fax: +39 0464483520

25 E-mail: alessandro.fornasiero@gmail.com

26 **Abstract**

27 The aims of the study were to describe the physiological profile of a 65-km (4000-m cumulative
28 elevation gain) running mountain ultra-marathon (MUM) and to identify predictors of MUM
29 performance. Twenty-three amateur trail-runners performed anthropometric evaluations and an
30 uphill graded exercise test (GXT) for $\text{VO}_{2\text{max}}$, ventilatory thresholds (VTs), power outputs
31 associated with these indices (PMax, PVTs) and heart rate response (HRmax, HR@VTs). Heart rate
32 (HR) was monitored during the race and intensity was expressed as: Zone I (<VT1), Zone II (VT1-
33 VT2), Zone III (>VT2) for exercise load calculation (training impulse, TRIMP). Mean race
34 intensity was $77.1\% \pm 4.4\%$ of HRmax distributed as: $85.7\% \pm 19.4\%$ Zone I, $13.9\% \pm 18.6\%$ Zone II,
35 $0.4\% \pm 0.9\%$ Zone III. Exercise load was 766 ± 110 TRIMP units. Race time (11.8 ± 1.6 h) was
36 negatively correlated with $\text{VO}_{2\text{max}}$ ($r = -0.66$, $P < 0.001$) and PMax ($r = -0.73$, $P < 0.001$), resulting these
37 variables determinant in predicting MUM performance, whereas exercise thresholds did not
38 improve performance prediction. Anthropometric and physiological variables explained only 59%
39 of race time variance, underlining the multi-factorial character of MUM performance. Our results
40 support the idea that VT1 represents a boundary of tolerable intensity in this kind of events, where
41 exercise load is extremely high. This information can be helpful in identifying optimal pacing
42 strategies to complete such extremely demanding MUMs.

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47 **Keywords:** *mountain ultra-marathon, heart rate, exercise intensity distribution, training load,*
48 *thresholds*

49 **Introduction**

50 Mountain ultra-marathons (MUMs) consist of running and walking on mountain trails over a
51 distance longer than the traditional marathon (from 42.2 up to 350 km) with a considerable
52 cumulative elevation gain (up to 25.000m). These events take place in mountain environments and
53 are performed on irregular terrain, presenting positive and negative slopes. Accordingly, to face
54 MUMs, athletes must perform prolonged concentric work against gravity force during ascents and
55 extensive eccentric work during downhill sections (Vernillo et al., 2015). In addition, MUMs
56 participants are exposed to multiple internal and external stressors, from exercise and environment,
57 including possible wide fluctuations in temperature and altitude, and generally have to sustain
58 extreme exercise loads (Millet, G. P. & Millet, 2012).

59 Because of their peculiarities some authors have suggested MUMs as an outstanding opportunity to
60 investigate the adaptive responses of the human body to the extreme load and stress of ultra-
61 endurance exercises (Millet, G. P. & Millet, 2012). Accordingly, recent studies have assessed the
62 acute consequences, as well as the adaptive responses induced by MUMs. MUMs have been
63 associated with musculoskeletal injuries and skin-related disorders (Vernillo et al., 2016b), negative
64 energy balance (Martinez et al., 2017; Ramos-Campo et al., 2016), severe muscular damage and
65 inflammation (Carmona et al., 2015; Saugy et al., 2013), marked neuromuscular fatigue (Easthope
66 et al., 2010; Millet, G. Y. et al., 2011b; Saugy et al., 2013), cardiac dysfunctions and myocardial
67 damage (Ramos-Campo et al., 2016; Vitiello et al., 2013), alterations in water diffusivity with
68 changes of the inter-cellular space at brain level (Zanchi et al., 2017), impairment in lung functions
69 (Vernillo et al., 2014a; Wuthrich et al., 2015) and in postural control (Degache et al., 2014). Besides
70 the acute consequences, recent studies reported physiological adaptations that seem to occur
71 exclusively following this specific ultra-endurance exercise. In particular specific metabolic
72 adaptation responses, like the reduction of running and walking uphill energy cost, have been
73 reported especially after extreme distance MUMs (Vernillo et al., 2016c; Vernillo et al., 2014b).

74 Despite the large number of investigations addressing the consequences of these extreme exercise
75 loads, limited information is available about the sustained exercise intensity and the physiological
76 demands faced during MUMs. The knowledge of the intensity profile and the physiological
77 requirements of MUMs can provide essential information for optimal training, nutrition and
78 participation, also considering the growing interest for these events, with annual numbers of races
79 and participants that are increasing considerably (Hoffman, Ong, & Wang, 2010).

80 Only few studies reported the intensity sustained during a MUM event. In a 54-km (≈ 2900 m d+) MUM
81 the mean intensity reported was 64% of maximal heart rate (HR_{max}) for the ≈ 14 h of its
82 duration (Clemente-Suarez, 2015). Conversely, the mean intensity of 82% of HR_{max} was reported
83 in athletes completing a 54-km (2700 d+) MUM in ≈ 7 h (Ramos-Campo et al., 2016). Despite
84 measuring two MUMs with similar characteristics, the mean exercise intensity was markedly
85 different between the two studies, thus making the scenario not clear. Moreover, the lack of a
86 description of participants' exercise capacities does not help the understanding of the elevated time-
87 difference observed in MUMs, that can be related to differences in performance level as trained
88 athletes are typically able to sustain higher exercise intensities for prolonged periods of time (Joyner
89 & Coyle, 2008; Lucia, Pardo, Duran, Hoyos, & Chicharro, 1998), but also the differences in
90 athletes' motivation in competing or simply being able to complete such extremely demanding
91 races.

92 In this regard, a detailed analysis of MUM participants' characteristics would certainly enhance the
93 comprehension of the determinants of MUM performance, where many factors have been shown to
94 be involved (Millet, G. Y., Hoffman, & Morin, 2012). In addition MUMs competitions can present
95 large withdrawal rates (Wegelin & Hoffman, 2011). Among the reasons for the considerable drop
96 out in MUMs inadequate pacing strategies (i.e. choice of exercise intensity) must be certainly
97 considered.

98 In the light of these observations, further investigations seem to be required to characterize the
99 exercise intensity sustained during MUMs, as well as how athletes' efforts are distributed among
100 the intensity spectrum for this kind of ultra-endurance exercise. Accordingly, the aim of the study
101 was to measure the sustained intensity during a 65-km MUM, characterizing the effort on the basis
102 of well-defined exercise intensity thresholds and quantifying the physiological load associated with
103 the competition. The second aim was to identify predictors of MUM performance by means of
104 multiple regression analysis between standardized laboratory testing measures (predictors) and race
105 time (dependent variable).

106 **Methods**

107 *Participants*

108 Twenty-three recreational healthy trail-runners (age 40.2 ± 7.3 yr), 17 males and 6 females, were
109 recruited for the study through advertisements on the official website of the race. None of the
110 participants involved had clinical evidence of cardiovascular, neuromuscular, or articular diseases.
111 Information about subjects' training history was collected through a questionnaire (Vernillo et al.,
112 2016b). Participants had 7 ± 7 yrs of training experience in running and 3 ± 3 yrs of experience in
113 MUMs. Usually they ran 7 ± 3 h/week covering 55 ± 31 km weekly. They participated in the
114 competition with the aim to complete it in the best time possible. Before data collection, all
115 participants were properly informed about the experimental protocol and gave their written
116 informed consent for the measures. The experimental protocol was approved by the Ethics
117 Committee of the University the investigators belong to.

118 *Experimental Protocol*

119 The study was conducted in two phases consisting of preliminary laboratory testing and during-race
120 monitoring. This study examined the HR response during a 65-km MUM in relation with HR-based
121 intensity markers: maximal heart rate (HR_{max}), heart rate at the first and at the second ventilatory

122 threshold (HR@VT1, HR@VT2). All participants visited our laboratories within the two weeks
123 before the competition for the preliminary testing session. Athletes performed a measure of
124 anthropometric characteristics and an uphill running graded exercise test (GXT) to identify
125 physiological parameters, including $\text{VO}_{2\text{max}}$ and ventilatory thresholds (VT1, VT2), as well as the
126 HR response. Athletes were asked to refrain from caffeine, alcohol and heavy exercise on the day
127 before the tests. All tests were conducted under controlled conditions ($20 \pm 1^\circ\text{C}$, 40-60% relative
128 humidity).

129 *Anthropometric characteristics*

130 Body mass (BM), was measured to the nearest 0.1 kg with a digital weighing scale (Seca, Hamburg,
131 Germany). Height was measured to the nearest 0.001 m with a wall-mounted stadiometer (Gima,
132 Milan, Italy). Body composition was performed with plicometry method by an experienced
133 investigator. Skin-fold data were obtained using a skin-fold calliper (Gima, Milan, Italy) and
134 recorded to the nearest 0.2 mm. Measurements were taken twice, and a mean of the two measures
135 was used for body fat calculation. To calculate values of fat mass (FM) and free-fat mass (FFM),
136 the percentage of body fat (%BF) was estimate according to estimated equations (Jackson &
137 Pollock, 1978; Jackson, Pollock, & Ward, 1979).

138 *Graded exercise test*

139 An uphill graded exercise test (GXT), by means of power increments (combined increases of speed
140 and inclination), was conducted on a motorized treadmill (Rodby Innovation AB, Vänge, Sweden).
141 Mechanical power expressed (W/kg) was calculated as $[\text{Power} = g \cdot v \cdot \sin(\alpha)]$, where g was the
142 gravitational acceleration (m/s^2), v the belt speed (m/s) and α the angle of treadmill inclination.
143 Before the test, each athlete performed a 10 min warm-up at a constant power of 0.5 W/kg. The test
144 started at a workload of 0.5 W/kg with increments of 0.5 W/kg (0.3 W/kg for females) every 3 min
145 until the volitional exhaustion. Cardio-respiratory measures were collected continuously with

146 breath-by-breath method using an automated open-circuit gas analysis system (Quark PFT Ergo,
147 Cosmed Srl, Rome, Italy). HR was recorded continuously during the test by a HR monitor
148 incorporated into the gas analysis system. Careful calibrations of flow sensors and gas analyzers
149 were performed before each measurement according to the manufacturer's instructions.

150 *Competition measurements*

151 The competition was a 65-km MUM, the second edition of Vigolana Trail® (Vigolo Vattaro, TN,
152 Italy) and was held in the first week of June. It involved 4000 m of cumulative elevation gain. The
153 starting point and the finish line were at 725 m altitude. Overall, the race was performed at medium
154 altitude, with an altitude range between 725 and 2100 m. The race started at 6.30 am with a
155 temperature of 20 °C. The recorded temperatures (minimum-maximum) were 20-33 °C. Maximal
156 allowed time for the 65 km MUM was 15.5 hours and the winner completed it in 7.1 hours. 154
157 participants of 188 starters finished the race (82%) with a mean time of 11.3 ± 1.7 h.

158 During the race, HR was continuously monitored using portable HR monitors (Polar RS800 SD,
159 Polar Electro, Kempele, Finland) averaged at 5 s intervals. Racing VO_2 was estimated for every
160 subject from the HR responses, according to the equations for the linear relationship between
161 oxygen uptake and HR obtained during the GXT. Due to technical problems related to difficulties
162 of such long-distance events not all participants were successfully monitored during the whole race.
163 The main reason was the discomfort caused by the thoracic belt for HR recording. Thus only 12 (8
164 males) out of 23 participants' HR profiles were available for the analysis. The characteristics of this
165 sub-group were not significantly different from the whole group of the study, all p values were
166 >0.05 .

167 *Data Analysis*

168 The maximal power output (PowerMax), achieved at athlete's exhaustion, was determined
169 according to the equation: $\text{PowerMax (W/kg)} = \text{power output last stage completed (W/kg)} + [t$

(s)/step duration (s) * step increment (W/kg)], where t is the time of the uncompleted stage (Kuipers, Verstappen, Keizer, Geurten, & Van Kranenburg, 1985). $\text{VO}_{2\text{max}}$ was defined as the highest values of a 20-s average (Robergs, Dwyer, & Astorino, 2010). Other breath-by-breath data were averaged over 10s for further analysis of other physiological parameters that have been shown to be important determinants of performance in endurance exercise (Lucià, Hoyos, Paèrez, & Chicharro, 2000). The first and the second ventilatory thresholds (VT1 and VT2) were determined from visual inspection by two independent operators according to methods described in detail elsewhere (Ahmaidi et al., 1993; Wasserman, Hansen, Sue, Stringer, & Whipp, 1999). Therefore, it was possible to establish the specific heart rate (HR@VT1 and HR@VT2) and power values associated with these intensities. Exercise intensity distribution during the race was calculated using HR profile and expressed into three zones: Zone I (<VT1) low intensity, Zone II (VT1-VT2) moderate intensity, Zone III (>VT2) high intensity. Total exercise load was calculated by means of the time spent in the three zones multiplied by arbitrary weighting factors, according to Lucia's training impulse method (Lucia's TRIMP) (Lucia, Hoyos, Santalla, Earnest, & Chicharro, 2003). Accordingly, 1 min in Zone I was given a score of 1 TRIMP unit, 1 min in Zone II was given a score of 2 TRIMP units, and 1 min in Zone III was given a score of 3 TRIMP units. The total TRIMP score was obtained by combining the results of the three zones.

187 *Statistical Analysis*

188 All test data are presented as means \pm standard deviations (SD). All the data were tested for their
189 normal distribution (Shapiro–Wilk test). The relationships between performance and subjects
190 characteristics were analyzed using Pearson's correlation. To assess the relationship between
191 performance and laboratory variables we conducted a forward stepwise hierarchical multiple
192 regression analysis. We used performance time as dependent variable, and subjects' characteristics
193 as independent factors. Independent factors entered in four steps into the regression model in the
194 following order:

1. Anthropometry (Age, BMI and Body Fat)
2. Anthropometry + maximal values (PowerMax and VO_{2max})
3. Anthropometry + maximal values + values@VT2 (Power@VT2 and VO_2 @VT2)
4. Anthropometry + maximal values + values@VT2 + values@VT1 (Power@VT1 and VO_2 @VT1)

All statistical analysis was completed using a statistical software (SPSS Inc, Chicago, Illinois, USA). The level of statistical significance was set at $p < 0.05$.

Results

Descriptive statistics of preliminary laboratory testing were reported in Table 1. Mean race time for participants in the study was 11.8 ± 1.6 h (range 8.2-14.3 h), 11.5 ± 1.7 h (range 8.2-14.3 h) in HR monitored sub-group. Athletes performed the race at a mean intensity of 140.3 ± 8.6 bpm, $77.1 \pm 4.4\%$ of HRmax equal to $89.1 \pm 6.1\%$ of HR@VT1. Mean estimated VO_2 was $63.2 \pm 9.1\%$ of VO_{2max} .

*****Table1 about here*****

Representative example of HR response was reported in Figure 1.

*****Figure1 about here*****

HR distribution during the race was reported in Fig2a. During the race the exercise intensity distribution was: $85.7\% \pm 19.4\%$ Zone I, $13.9\% \pm 18.6\%$ Zone II, $0.4\% \pm 0.9\%$ Zone III (Fig2b). Total exercise load was 766 ± 110 TRIMP units. Correlations between laboratory variables and performance time were reported in Table2.

*****Figure2 about here*****

*****Table2 about here*****

217 Race time was negatively correlated with maximal physiological parameters, VO_{2max} ($r=-0.66$,
218 $P<0.001$) and PowerMax ($r=-0.73$, $P<0.001$), resulting these variables determinant in predicting
219 MUM performance. In contrast, despite the strong relationships observed with race time,
220 Power@VT2 ($r= -0.70$, $P<0.001$) and Power@VT1 ($r= -0.71$, $P<0.001$), sub-maximal parameters
221 associated with exercise thresholds did not improve performance prediction.

222 *****Figure3 about here*****

223 Results from multiple regression analysis were reported in Table 3.

224 *****Table3 about here*****

225 **Discussion**

226 *MUM exercise intensity*

227 Despite the high number of recent investigations performed on MUMs, limited information is
228 available about the sustained exercise intensity and the physiological demands of these events. Most
229 of the knowledge available on ultra-marathons is based on flat running performance, where
230 intensities have been reported to be 60%-70% of VO_{2max} in 6-h events (Davies & Thompson, 1979),
231 decreasing to 40%-50% of VO_{2max} in 24-h events (Millet, G. Y. et al., 2011a). Only few studies,
232 based on HR monitoring, reported the intensity sustained during MUMs. The mean intensities of
233 64% of HRmax and 82% of HRmax were respectively reported for participants completing a 54-km
234 MUM in ≈ 14 h (Clemente-Suarez, 2015) and ≈ 7 h (Ramos-Campo et al., 2016).

235 In our study the intensity observed, $\approx 77\%$ of HRmax, equal to an estimated intensity of $\approx 63\%$ of
236 VO_{2max} , was comparable to other ultra-endurance events of similar duration (≈ 10 -11h) (Barrero,
237 Chaverri, Erola, Iglesias, & Rodriguez, 2014; Laursen et al., 2005). In ultra-endurance triathlons
238 mean intensities observed were 78% (Barrero et al., 2014) and 83% (Laursen et al., 2005) of
239 HRmax during cycling and 77% HRmax during running (Barrero et al., 2014; Laursen et al., 2005).

240 Differently, for events of longer duration lower HR values have been usually observed together
241 with a decrease of intensity with time (Gimenez, Kerhervè, Messonnier, Fèasson, & Millet, 2013;
242 Neumayr, Pfister, Mitterbauer, Maurer, & Hoertnagl, 2004). Gimenez and colleagues (2013)
243 observed a decrease from 72% to 62% of HRmax between the first to the last 6 h of a 24-h treadmill
244 running, with mean intensity sustained of 68% of HRmax (Gimenez et al., 2013). Accordingly, our
245 results obtained during a MUM event seem to be in line with other studies on ultra-endurance
246 exercise.

247 To the best of our knowledge this is the first investigation analyzing the exercise intensity
248 distribution during a MUM, characterizing the effort by means of well-defined exercise thresholds
249 (VTs). In previous mentioned investigations (Clemente-Suarez, 2015; Ramos-Campo et al., 2016)
250 MUM exercise intensity was found to be below the onset of blood lactate accumulation (OBLA),
251 however no evaluation tests were conducted in order to characterize athletes' effort continuously
252 during the competition. According to exercise intensity distribution found in this investigation most
253 of the race was spent in Zone I, below HR@VT1 (Fig2b). In line with our findings, previous
254 authors have suggested that the intensity associated with VT1 cannot be maintained throughout an
255 ultra-endurance event (Laursen et al., 2005), showing that in the running phase of ultra-endurance
256 triathlons athletes performed below HR@VT1 (Barrero et al., 2014; Laursen et al., 2005).
257 Accordingly, in ultra-endurance exercise the existence of an ultra-endurance threshold lower than
258 VT1 and 80% of HRmax has been previously proposed (Laursen et al., 2005; O'Toole, Douglas, &
259 Hiller, 1998). In our study the mean exercise intensity maintained was slightly below
260 90%HR@VT1. It has been suggested that exercise intensities marginally below VT1 allow a better
261 balance of substrates oxidation, promoting higher fat to carbohydrate utilization, sparing
262 carbohydrate reserves, delaying muscle and liver glycogen depletion, and maintaining blood
263 glucose concentration (Barrero et al., 2014; Laursen et al., 2005; Laursen & Rhodes, 2001). This
264 strategy has been recommended to help ultra-endurance athletes in reducing fatigue and improving
265 performance (Laursen & Rhodes, 2001). Moreover, during ultra-endurance events athletes present

large energy expenditures and require constant energy refuelling (Jeukendrup, 2011; Kreider, 1991). Particularly, despite nutritional strategies adopted by the athletes, MUMs competitions, are associated with large energy deficits (Martinez et al., 2017; Ramos-Campo et al., 2016). Thus, the adoption of an optimal exercise intensity, together with an adequate nutritional intake (Jeukendrup, 2011; Martinez et al., 2017), probably represent the best solution to delay the onset of fatigue and compete in MUMs. Accordingly, an intensity slightly lower than VT1 could represents a boundary of sustainable intensity for runners in >10h MUMs, since athletes sustaining a large part of the race in Zone I could manage their energy reserves, avoid nutrient-related fatigue and optimize competitive result. This information observed in runners that successfully completed a 65-km MUM can be helpful for athletes and coaches in order to better plan the trainings and the participation in this kind of events. In particular our findings can help athletes' pacing strategy during MUMs competitions, providing a reference threshold for athletes who aim to complete such extreme races.

MUM exercise load

The three zones approach defining exercise intensity by means of the HR at the two ventilatory thresholds has been extensively used to calculate the exercise load of trainings and competitions (TRIMP), as well as the optimal training intensity distribution, both in endurance and ultra-endurance athletes (Muñoz, Cejuela, Seiler, Larumbe, & Esteve-Lanao, 2014; Seiler & Kjerland, 2006; Stöggl & Sperlich, 2015). HR-based TRIMP score in literature showed training loads of ≈ 1000 -1500 TRIMP units/week in professional cycling (Lucia et al., 2003) , ≈ 1000 units/week in ultra-endurance tri-athletes (Muñoz et al., 2014), ≈ 800 units/week elite runners (Billat et al., 2003), ≈ 400 units/week sub-elite runners (Esteve-Lanao, San Juan, Earnest, Foster, & Lucia, 2005) and ≈ 800 units/week in elite junior Nordic skiers (Seiler & Kjerland, 2006). Moreover, taking into account competition loads, values of ≈ 2000 TRIMP units/week during professional road cycling competition (Lucia, Hoyos, Carvajal, & Chicharro, 1999), ≈ 1000 units during Ironman triathlon

291 (Muñoz et al., 2014) and ≈ 800 units during a 24-h cycling race were reported (Bescos et al., 2012).
292 In the light of the above, the ~ 750 TRIMP units observed in this study can be considered extremely
293 high, especially for amateur athletes, as such values are often reached by endurance athletes during
294 an entire week of training.

295 *MUM performance*

296 The 65-km MUM performance was highly correlated with athletes' $\text{VO}_{2\text{max}}$ and peak power output
297 reached in the graded exercise test (Fig. 3). By including the oxygen consumption and mechanical
298 power exerted at the ventilatory thresholds, despite being highly correlated with MUM
299 performance, the prediction of race time did not improve (see the results of steps 3 and 4 of
300 hierarchical regression analysis reported in Table 3). Considering the submaximal intensities
301 sustained in MUMs, it was plausible that the oxygen consumptions associated with sub-maximal
302 indices ($\text{VO}_2@V\text{T}_s$) represented parameters able to predict the performance. Particularly, for
303 endurance exercise, submaximal indices (e.g. power output or speed exerted at the ventilatory
304 thresholds) seem to be more reflective of athletes' performance capability (Impellizzeri, Marcora,
305 Rampinini, Mognoni, & Sassi, 2005; Lucia et al., 1998), as well as better descriptive of training
306 status especially in an homogenous group of athletes (e.g. similar $\text{VO}_{2\text{max}}$) (Joyner & Coyle, 2008).
307 Nevertheless for ultra-endurance exercises values associated with these intensity markers seem to
308 be not so determinant (Millet, G. Y. et al., 2011a), resulting maximal values the best performance
309 predictors (Barrero et al., 2014). In line with existing ultra-endurance literature our analysis,
310 conducted in a heterogeneous group of athletes, further showed the importance of maximal values
311 over those associated with exercise thresholds in ultra-endurance exercise, as previously reported
312 for ultra-distance running (Millet, G. Y. et al., 2011a; Millet, G. Y. et al., 2012) and ultra-endurance
313 triathlon (Barrero et al., 2014). In particular, $\text{VO}_{2\text{max}}$ is still associated with performance also in
314 ultra-endurance events up to 24-h in duration (Lazzer et al., 2012; Millet, G. Y. et al., 2011a). The
315 importance of a high $\text{VO}_{2\text{max}}$ has been also explained by a favorable metabolic condition, connected

316 with an advantageous substrates utilization, during low intensities observed in ultra-endurance
317 exercises (Millet, G. Y. et al., 2011a). In this regard, high values of $\text{VO}_{2\text{max}}$ could represent also a
318 beneficial aspect for the sub-maximal intensities and long duration of a MUM.

319 In the present study the power outputs exerted in graded exercise test, calculated at the level of
320 ventilatory thresholds and $\text{VO}_{2\text{max}}$, were better correlated with performance (r coefficients ranged
321 from -0.73 to -0.71) than the measure of oxygen consumptions at the same intensities (r coefficients
322 ranged from -0.66 to -0.56, see Table 2). Differently from the measure of oxygen consumptions, the
323 measurement of external power output takes into account the efficiency of converting metabolic
324 power in mechanical power (Ettema & Loràs, 2009), representing one of the main determinants of
325 endurance performance (Joyner & Coyle, 2008). Thus, the power output that an athlete can produce,
326 determined by an uphill GXT, may represent an important factor, determining the ascent rate and
327 consequently performance time in uphill sections of a MUM.

328 The variables derived from anthropometry and a GXT were found to explain only the 59% of MUM
329 performance variance. In this regard, in ultra-distance running events other factors, associated with
330 the extreme character of the races, as the resistance to muscle damage and mental abilities, can play
331 an important role in determining the final result (Millet, G. Y. et al., 2012). In addition an
332 extensively investigated variable in ultra-distance running that was not evaluated in this study is
333 energy cost of locomotion (Lazzer et al., 2012; Millet, G. Y. et al., 2011a; Vernillo et al., 2016c;
334 Vernillo et al., 2015; Vernillo et al., 2014b). The role of energy cost in determining ultra-running
335 performance is still a topic of discussion (Millet, G. Y. et al., 2012). Previous authors have shown
336 that mean energy cost of level running together with $\text{VO}_{2\text{max}}$ and its fractional utilization can
337 explain the 87% of performance in multi-day running (Lazzer et al., 2012). In addition, as acute
338 consequence of MUM participation, changes in energy cost in different running conditions have
339 been reported (Vernillo et al., 2016c; Vernillo et al., 2015; Vernillo et al., 2014b), with variations
340 that have been shown to be related to MUM performance (Vernillo et al., 2015). For instance,

341 Vernillo and colleagues (Vernillo et al., 2015) reported a positive correlations between race time
342 and the energy cost variation in level and uphill running, after a previous edition of this MUM (65-
343 km). In this study we did not measure the energy cost in different running conditions, and its
344 variation after the race, this may explain why anthropometric and physiological characteristics
345 measured with a GXT accounted only for the 59% of MUM performance variance. Accordingly,
346 these results and the factors above mentioned can further underline the multi-factorial character of
347 MUM performance (Millet, G. P. & Millet, 2012).

348 *Limitations*

349 Some issues should be considered when interpreting the present results. The long distance, the
350 alternation of high elevation gain and loss of the MUM may have favoured the use of conservative
351 pacing strategies, decreasing the risk of premature exhaustion. In addition, several factors might
352 have influenced the HR response during the MUM. The effect of altitude (Bartsch & Gibbs, 2007)
353 as well as subjects' hydration status (Lambert, Mbambo, & Gibson, 1998) could have indeed caused
354 increases in HR. Furthermore, reductions in HR have been observed after ultra-endurance exercise
355 (Lucas et al., 2008; Mattsson et al., 2010) due to plasma volume expansion (Robach et al., 2014)
356 and the desensitization of the heart's adrenergic receptors (Hart et al., 2006; Welsh et al., 2005).
357 The downhill sections of the MUM, generating more exercise-induced muscle damage and fatigue-
358 related outcomes (Giandolini et al., 2016), may have played a direct role on the physiological load
359 not considered in the study. If the athletes stayed for most of the time at an intensity < HR@VT1
360 during downhill sections the physiological stress may have been quite blind by the intrinsic features
361 of the downhill locomotion (Giandolini et al., 2016; Minetti, Moia, Roi, Susta, & Ferretti, 2002;
362 Vernillo et al., 2016a). Nevertheless, prolonged eccentric loads can lead to an increase of the
363 oxygen consumption, mainly related to the exercise-induced muscle damage (Giandolini et al.,
364 2016; Vernillo et al., 2016a), and thus in the physiological strain. In this regard GPS data could be

365 helpful to contextualize the different contribution of uphill and downhill sections and, thus, the
366 physiological load of MUMs (Kerhervè, Millet, & Solomon, 2015).

367 **Conclusions**

368 Mean exercise intensity during the 65-km MUM was $\approx 77\%$ of HR_{max} and most of the race time
369 was spent at intensity below HR@VT1. This finding supports the idea that the first ventilatory
370 threshold represents a boundary of tolerable intensity for amateur runners in a MUM longer than
371 10h, where the exercise load was found to be extremely high (>750 TRIMP units). The results can
372 be helpful for athletes and coaches in order to better plan the training strategies and the participation
373 in this kind of events. In particular our findings can help athletes' pacing strategy during MUMs
374 competitions, providing a reference threshold for athletes who aim to complete such extreme races.

375 In addition, the study showed that parameters associated with $\text{VO}_{2\text{max}}$ were determinant in
376 predicting MUM performance, whereas exercise thresholds did not improve performance prediction
377 in this heterogeneous group of athletes, which is in line with previous research in ultra-endurance
378 events. However, the variables derived from anthropometry and a graded exercise test explained
379 only 59% of race time variance, further underlining the multi-factorial character of MUM
380 performance.

381 **Disclosure of interest**

382 The authors report no conflicts of interest.

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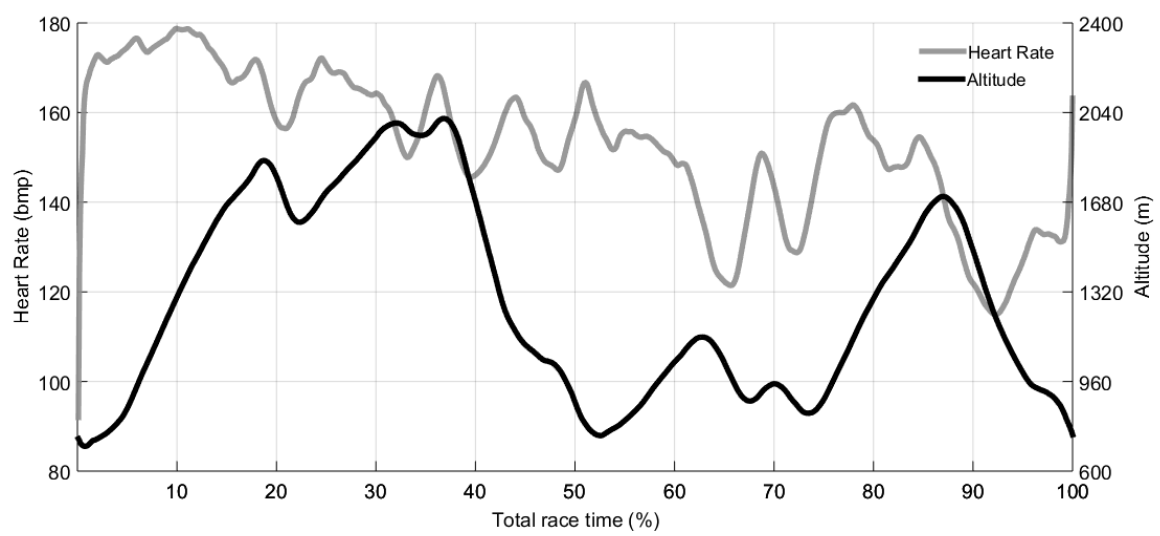
573 **Figures captions**

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576 **Figure 1.** Heart rate response (bpm) and change in altitude (m) during the MUM expressed as % of
577 total race time in a representative participant.

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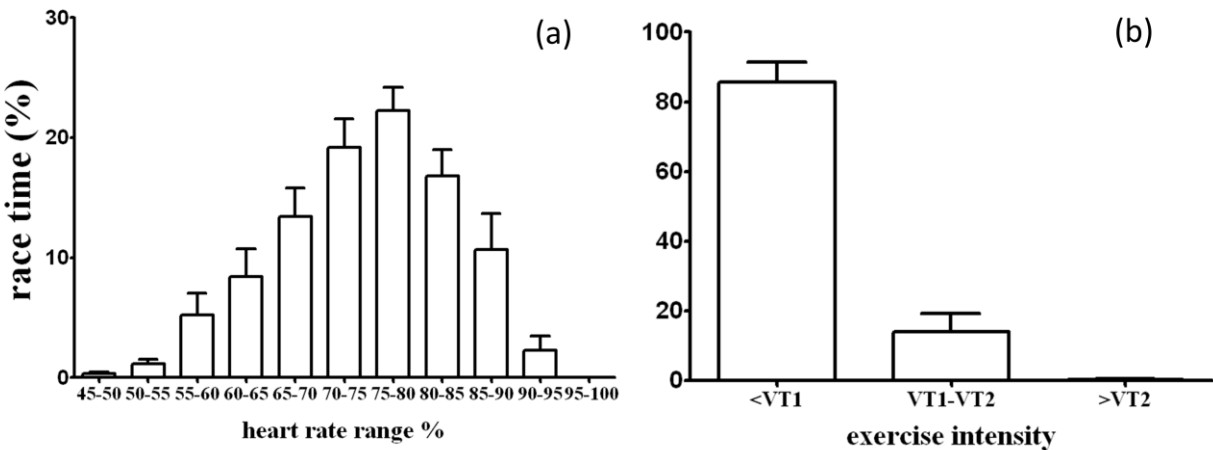
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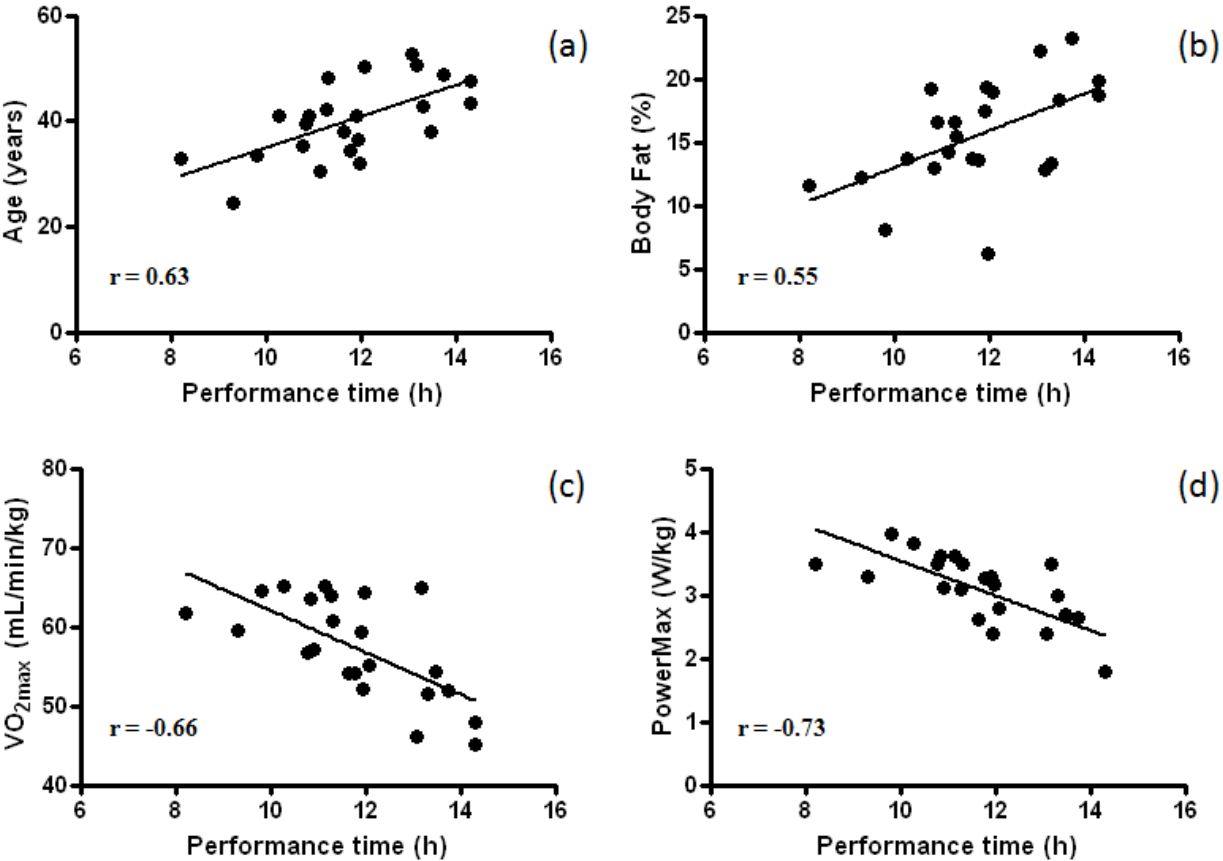
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584 **Figure 2. (a)** Heart rate distribution during the race. Time spent at different ranges of maximal heart
585 rate expressed as % of total race time. **(b).** Exercise intensity distribution during the race. Time
586 spent in Zone 1 (<VT1), Zone 2(VT1-VT2), Zone 3 (>VT2) expressed as % of total race time.
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602 **Figure 3.** Correlations with performance time in MUM (a) Age (years) (b) Body Fat (%) (c)
603 $\text{VO}_{2\text{max}}$ (mL/min/kg) (d) Maximal power in uphill graded exercise test



617 **Table 1.** Characteristics of the participants resulting from preliminary laboratory testing session.

Characteristics of the subjects										
	Whole group (n=23)					Subgroup HR monitored (n=12)				
	mean	±	s.d	range		mean	±	s.d	range	
Age (years)	40.2	±	7.3	24.4	- 52.7	38.6	±	6.1	30.4	- 48.9
Anthropometry										
Body mass (kg)	69.2	±	11.8	47.0	- 86.1	65.8	±	12.1	47.0	- 83.5
Height (cm)	173	±	8	157	- 187	171	±	9	157	- 181
BMI (kg/m ²)	22.9	±	2.5	18.8	- 27.3	22.2	±	2.7	18.8	- 27.3
Fat-free mass (kg)	58.4	±	9.9	39.6	- 73.0	55.8	±	10.5	39.6	- 68.8
Fat mass (kg)	10.8	±	3.8	3.9	- 19.4	10.0	±	4.1	3.9	- 19.4
Body fat (%)	15.6	±	4.2	6.2	- 23.3	15.1	±	5.0	6.2	- 23.3
Graded exercise test										
VO _{2max} (ml/min/kg)	57.4	±	6.3	45.2	- 65.1	58.4	±	6.2	48.0	- 65.1
VO ₂ @VT2 (ml/min/kg)	51.9	±	5.5	40.3	- 59.5	52.9	±	5.0	45.5	- 59.5
VO ₂ @VT1 (ml/min/kg)	45.3	±	5.1	33.0	- 52.1	46.3	±	4.5	36.8	- 52.1
HRmax (bpm)	181	±	8	166	- 196	182	±	8	166	- 196
HR @VT2 (bpm)	169	±	10	150	- 186	171	±	10	154	- 186
HR @VT1 (bpm)	155	±	11	128	- 175	158	±	11	136	- 175
PowerMax (W/kg)	3.1	±	0.6	1.8	- 4.0	3.1	±	0.6	1.8	- 4.0
Power@VT2 (W/kg)	2.3	±	0.5	1.4	- 3.0	2.4	±	0.4	1.6	- 3.0
Power@VT1 (W/kg)	1.7	±	0.4	1.0	- 2.2	1.7	±	0.3	1.0	- 2.2
Performance										
Total race time (h)	11.8	±	1.6	8.2	- 14.3	11.5	±	1.7	8.2	- 14.3

VO_{2max} : maximal oxygen consumption; VO₂ @VTs: oxygen consumption at ventilatory thresholds;
HRmax: maximal heart rate; HR @ VTs: heart rate at ventilatory thresholds; PowerMax: maximal
mechanical power output; Power @VTs: power output at the ventilatory thresholds

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620 **Table 2.** Relationship between participants' anthropometric and physiological characteristics and
621 MUM performance (race time).

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Performance Correlation Analysis				
(n=23)				
	r	90% CI		p
Age (years)	0.63	0.44	0.77	<0.001
Anthropometry				
BMI (kg/m ²)	0.07	-0.27	0.40	0.384
Fat-free mass (kg)	-0.26	-0.56	0.08	0.112
Fat mass (kg)	0.40	0.12	0.64	0.028
Body fat (%)	0.55	0.29	0.76	0.004
Graded exercise test				
VO _{2max} (ml/min/kg)	-0.66	-0.83	-0.44	<0.001
VO ₂ @VT2 (ml/min/kg)	-0.65	-0.74	-0.35	<0.001
VO ₂ @VT1 (ml/min/kg)	-0.56	-0.83	-0.44	0.003
PowerMax (W/kg)	-0.73	-0.87	-0.56	<0.001
Power@VT2 (W/kg)	-0.70	-0.87	-0.46	<0.001
Power@VT1 (W/kg)	-0.71	-0.90	-0.45	<0.001

VO_{2max} : maximal oxygen consumption; VO₂ @VTs: oxygen consumption at ventilatory thresholds; HRmax: maximal heart rate; HR @ VTs: heart rate at ventilatory thresholds; PowerMax: maximal power output; Power @ VTs: power output at ventilatory thresholds

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633 **Table 3.** Model Summary resulting from forward stepwise hierarchical multiple regression analysis.

Model	Coefficients B	90% CI for B		Standardized Coefficients	Sig.	Partial R	R	R ²	Adjusted R ²	R ² Change	Sig. Change
		Lower	Upper								
1 (Constant)	7.844	3.323	12.365		0.007						
Age	0.103	0.026	0.180	0.481	0.032	0.470	0.682	0.465	0.381	0.465	0.007
BMI	-0.088	-0.279	0.103	-0.142	0.435	-0.180					
Body Fat	0.116	-0.023	0.255	0.311	0.166	0.313					
2 (Constant)	13.961	7.437	20.486		0.002						
Age	0.097	0.033	0.160	0.451	0.016	0.542	0.827	0.684	0.591	0.219	0.011
BMI	0.025	-0.150	0.200	0.040	0.808	0.060					
Body Fat	-0.057	-0.216	0.103	-0.151	0.545	-0.148					
VO _{2max}	-0.016	-0.155	0.123	-0.065	0.843	-0.049					
PowerMax	-1.583	-2.898	-0.268	-0.593	0.052	-0.453					
3 (Constant)	14.479	7.449	21.509		0.003						
Age	0.096	0.029	0.163	0.448	0.024	0.543	0.834	0.696	0.554	0.012	0.743
BMI	0.007	-0.185	0.199	0.011	0.950	0.016					
Body Fat	-0.046	-0.217	0.124	-0.124	0.640	-0.122					
VO _{2max}	0.135	-0.258	0.529	0.547	0.555	0.154					
PowerMax	-2.591	-5.449	0.267	-0.971	0.133	-0.380					
VO ₂ @VT2	-0.170	-0.577	0.236	-0.595	0.474	-0.186					
Power@VT2	1.279	-2.027	4.585	0.379	0.508	0.172					
4 (Constant)	15.641	7.974	23.309		0.003						
Age	0.099	0.032	0.166	0.464	0.021	0.589	0.869	0.756	0.587	0.060	0.242
BMI	0.052	-0.141	0.245	0.084	0.640	0.132					
Body Fat	-0.124	-0.315	0.068	-0.331	0.273	-0.303					
VO _{2max}	0.156	-0.243	0.554	0.630	0.501	0.189					
PowerMax	-3.714	-6.726	-0.701	-1.391	0.048	-0.518					
VO ₂ @VT2	-0.446	-1.105	0.213	-1.559	0.252	-0.316					
Power@VT2	5.020	0.072	9.968	1.487	0.096	0.446					
VO ₂ @VT1	0.268	-0.088	0.625	0.865	0.206	0.347					
Power@VT1	-3.272	-6.885	0.341	-0.744	0.133	-0.406					

634 Dependent Variable: Performance time (h)